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Short-term feeding of vitamin D₃ improves color but does not change tenderness of pork-loin chops¹

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ABSTRACT: The objective of this study was to determine the effect of short-term feeding of vitamin D₃ (D₃) on blood plasma calcium concentrations and meat quality of pork-loin chops. Three experiments were carried out to meet this objective. Experiment 1 used 250,000 IU and 500,000 IU/d to determine the effective dose of dietary D₃ to raise blood plasma calcium concentration. Experiment 2 used 500,000 IU D₃/d to determine the appropriate length of feeding time to elevate blood plasma calcium prior to harvest. Experiment 3 used 500,000 IU D₃/d to determine the effectiveness of increased blood plasma calcium in improving postmortem quality and tenderness of pork-loin chops. Pigs fed 500,000 IU D₃/d in Exp. 1 exhibited higher ($P < 0.05$) and more stable plasma calcium concentration over a 14-d feeding trial compared with pigs fed 250,000 IU

D₃/d and control pigs. Therefore, 500,000 IU D₃/d was the dose chosen for Exp. 2, in which pigs fed 500,000 IU D₃/d for 3 d prior to harvest exhibited elevated and stable plasma calcium concentrations; this length of time was deemed sufficient in which to observe differences in postmortem meat tenderness in Exp. 3. Vitamin D₃ supplementation resulted in lower ($P < 0.02$) L* values and higher ($P < 0.03$) a* values of loin chops at 7 and 14 d of shelf storage. Vitamin D₃ supplementation did not affect quality characteristics (measured by use of subjective scores) or tenderness (quantified via Warner-Bratzler shear force or Star probe values). On the basis of these findings, feeding 500,000 IU D₃/d to finishing pigs improved most Hunter color values at 14 d of storage but did not improve pork-loin chop tenderness at 1 to 21 d of retail shelf storage.

Key Words: Calcium, Cholecalciferol, Pork, Tenderness

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Introduction

There has been a renewed interest in pork tenderness because an increasingly tough pork product has been developed with the move toward higher lean growth genetics (Meisinger and Miller, 1998). With this change in palatability of pork, it seems relevant to investigate methods for increasing tenderness of fresh pork-loin chops.

Other meat species, including lamb and beef, also have meat tenderness problems (Koohmaraie et al., 1995; Miller et al., 1996). The association of calcium with meat tenderness is well defined (Koohmaraie et al.,

1990; Morgan et al., 1991; Whipple and Koohmaraie, 1992). Increasing muscle calcium increases the activity of calpains, which are intracellular proteases responsible for postmortem meat tenderness (Huff-Lonergan et al., 1996).

Recently, researchers investigated the efficacy of feeding high amounts of vitamin D₃ to increase postmortem muscle calcium and subsequently to improve meat tenderness (Swanek et al., 1999; Montgomery et al., 2000; Wiegand et al., 2000). These experiments resulted in an increase in beef tenderness (Swanek et al., 1999; Montgomery et al., 2000) but no change in callipyge lamb tenderness (Wiegand et al., 2000). The hypothesis in the current study was that feeding 250,000 or 500,000 IU of vitamin D₃ per day to finishing pigs for 3 d prior to slaughter would elevate plasma calcium and subsequently improve meat tenderness. To test this hypothesis, we conducted three experiments of feeding vitamin D₃ to finishing pigs. Experiment 1 was conducted to determine the appropriate dose, 250,000 or 500,000 IU per day, to observe an increase in plasma calcium concentrations. Experiment 2 was conducted to determine the appropriate length of time for feeding vitamin D₃ to maintain elevated plasma

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calcium at slaughter. Experiment 3 combined the effective dose with the effective length of feeding to determine the impact of feeding supplemental vitamin D₃ to pigs on pork-loin quality and tenderness.

Materials and Methods

Experiment 1

Eight finishing pigs (117 kg) were allotted randomly to groups fed 250,000 or 500,000 IU of vitamin D₃ daily for 7 d or until feed intake decreased. Pigs were penned individually and fed 2.5 kg of feed per day to ensure consumption of the vitamin D₃. Blood samples were drawn daily via jugular puncture for 8 d and every other day until d 14 for plasma calcium assay. Blood samples were collected in heparinized tubes and spun in a clinical centrifuge (Model CL IEC/Damon, Needham, MA) at 1500 × *g* for 15 min. Plasma was pipetted into glass vials and frozen at -30°C. At time of analysis, samples were thawed in a warm water bath (Isotemp; Fisher Scientific, Saint Louis, MO) and vortexed to ensure a homogenous sample. Blood plasma was pipetted into a solution containing lanthanum oxide (Sigma-Aldrich Co., Saint Louis, MO) to prevent interference by other metal cations and assayed for calcium concentrations by atomic absorption spectrophotometry (Perkin Elmer, Norwalk, CT; Willis, 1960). Calcium values are reported as milligrams of calcium per 100 mL of plasma.

Experiment 2

Twelve finishing pigs (115 kg) were fed 500,000 IU of vitamin D₃ daily for 1, 2, or 3 d. Pigs were penned individually and fed 2.5 kg of feed to ensure consumption of the vitamin D₃. Daily blood samples were taken before feeding for 7 d to monitor plasma calcium concentrations as described previously.

Experiment 3

Twenty-four finishing barrows (117 kg) were allotted randomly by litter to a control diet or a diet containing 500,000 IU of vitamin D₃ for 3 d. Pigs were fed 2.5 kg per day to ensure total feed consumption. On d 4, all pigs were given the control diet for 6 h and then transported to the Iowa State University Meat Laboratory for next-day slaughter. Blood samples were drawn before the feeding trial and 1 h before slaughter to determine plasma calcium concentrations. Carcasses were weighed before chilling. At 60 and 90 min after stunning, color of the longissimus was measured by using a Minolta colorimeter (Model CR-310; Minolta, Tokokawa, Japan). This measurement was taken between the 10th and 11th rib interface at the longissimus muscle of the ribbed carcass.

At 24 h postmortem, chilled carcass weight and pH of the longissimus were recorded. Longissimus pH was

determined by using a 10-g pulverized muscle sample homogenized in 90 mL of deionized water. Muscle samples were cut from the exposed loin face of the ribbed carcass between the 10th and 11th rib. The homogenate was filtered through a Whatman 125-mm paper and measured on an Accumet 125 pH meter (Fisher Scientific, Pittsburgh, PA). Subjective quality scores for color, marbling, and firmness were made at the 10th and 11th rib junction (NPPC, 1991). Quality measurements were determined by three individuals, each trained using National Pork Producers Council standards. Carcass measurements were taken for loin eye area at the 10th and 11th rib and for fat depth over the loin eye 3/4 the distance curvilinear at the 10th rib and at the last rib perpendicular to the longissimus muscle. Carcass measurements were the mean value from three trained persons.

Carcasses subsequently were fabricated into primal cuts, including ham (IMPS 401A), loin (IMPS 410), belly (IMPS 408), picnic shoulder (IMPS 405), and Boston butt (IMPS 406). The loin was deboned (IMPS 412B), and 2.54-cm chops were removed for simulated retail shelf-storage, sensory, and tenderness analysis. Chops were paired and placed in Viskase vacuum bags. At the appropriate day of storage (1, 7, 14, or 21) chops were placed on Styrofoam trays with oxygen-permeable polyvinyl overwrap. All chops were stored in a self-service case at 2°C.

At each storage day, chops were measured for color (1 h bloom time), pH, water-holding capacity (**WHC**), Warner-Bratzler shear force, and Star probe force. Color measurements were obtained by using a Hunter Labscan (Hunter and Associates, Reston, VA) with a 1.25-cm aperture, a D65 light source, and a 10° observer. Values for L*, a*, and b* were recorded. The pH values were determined by the previously described method. Water-holding capacity was measured by using the Carver Press Method (Kauffman et al., 1986). This method used a 0.3-g sample pressed onto an oven-dried Whatman 125-mm filter paper at 3000 psi. The WHC values were calculated as the ratio of the area of expressed water to the area of the pressed meat sample as measured with a planimeter (Model 4236; Keuffel and Esser, Hoboken, NJ). Therefore, a lower ratio indicates a greater WHC.

Loin chops that had been aged for 1, 7, 14, or 21 d were cooked to an internal temperature of 35°C and then turned and cooked to a final internal temperature of 71°C in a broiler oven (Model CN02; General Electric, Chicago Heights, IL) set at 177°C. Chops were cooled overnight at 4°C to ensure consistent internal temperature, and three 1.27-cm-round cores were removed parallel to the muscle fiber orientation from each chop. Warner-Bratzler shear force was determined with an Instron Testing Machine (Model 4502; Canton, MA). Cores were sheared with a Warner-Bratzler attachment perpendicular to the muscle fibers at a crosshead speed of 250 mm/min.

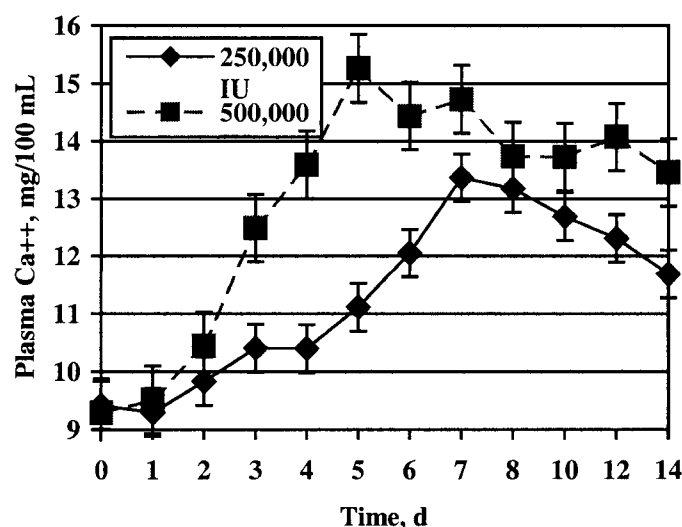


Figure 1. Plasma calcium concentration as affected by dosage of dietary vitamin D₃. Vitamin D₃ was fed on d 1 through 7.

Star probe puncture force also was measured on cooked chops with an Instron Universal Testing Machine (Instron, Canton, MA) (Oltrogge and Prusa, 1987; Malek et al., 2001). This procedure used a 105-mm steel probe with a 5-point star configuration. The probe was lowered to the surface of a cooked chop, and then the peak force for the probe to puncture 80% of the depth of the chop was recorded in kilograms.

Statistical Analysis

A completely randomized design with blocking by litter was used for this experiment. Data were analyzed by using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model included the main effect of diet, and repeated measures were used to analyze plasma calcium and color characteristics over time. A value of $P < 0.05$ was used to determine significance between main effect means. Data are presented as least squares means with the appropriate P -values attached.

Results and Discussion

Experiment 1

The objective of this experiment was to determine whether feeding 250,000 IU or 500,000 IU of vitamin D₃ per day to pigs would effectively raise plasma calcium concentrations. On the basis of data in Figure 1, we observed that feeding 500,000 IU per day raised plasma calcium to approximately a maximum of 15.0 mg/100 mL plasma by 5 d of feeding the vitamin D₃, which is a 39% increase, whereas the 250,000 IU per day resulted in only 11.0 mg/100 mL by d 5 of the trial. Cessation of feeding supplemental vitamin D₃ to pigs previously fed 250,000 IU/d and to those previously fed 500,000 IU/d resulted in decreases in plasma calcium

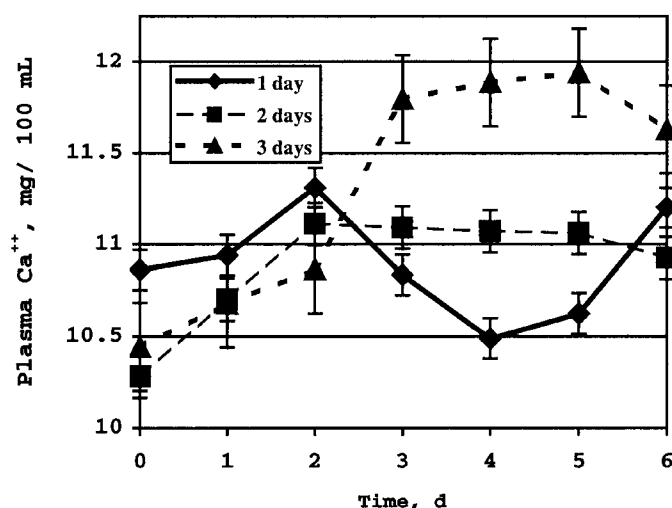


Figure 2. Plasma calcium concentration as affected by dosage of dietary vitamin D₃. Vitamin D₃ was fed on d 1 through 7.

from d 7 to d 14. The 500,000-IU dose caused plasma calcium to remain elevated above 13.0 mg/100 mL until d 14. In contrast, the 250,000-IU dose caused a peak of 13.2 mg/100 mL (30% increase), which did not occur until d 7. Plasma calcium decreased to 11.7 mg/100 mL at d 14 for pigs given the lower dosage of vitamin D₃. At a plasma calcium concentration above 13 mg/100 mL, feed intake of several pigs diminished slightly (data not shown), which probably contributed to the maximization of plasma calcium at 5 d rather than at 7 d for the 500,000 IU group. Presumably, the hypercalcemia of the pigs to curtail appetite is a physiological and protective response to prevent organ and bone damage (Jones et al., 1998). According to our working hypothesis that hypercalcemia is needed for vitamin D₃ to improve pork tenderness and that the larger dosage caused only minimal appetite suppression, we chose the 500,000 IU dosage of vitamin D₃ for Exp. 3.

Experiment 2

The objective of Exp. 2 was to determine the optimal number of days to feed 500,000 IU D₃ daily to achieve and maintain elevated plasma calcium concentrations until slaughter. The data in Figure 2 illustrate the change in plasma calcium concentrations as a function of time when this dose of vitamin D₃ was fed for 1, 2, or 3 d. These data show that 3 d of feeding vitamin D₃ resulted in the maximal plasma calcium concentration that was maintained for the longest time period after cessation of vitamin D₃ feeding. The plasma calcium concentration remained constant for approximately 3 d after removal of the vitamin D₃ supplement. If dietary vitamin D₃ supplementation proved to be effective in improving pork tenderness because of being hypercalcemic at death, pork producers would have a 3-d period after cessation of vitamin D₃ feeding in which to harvest

Table 1. Plasma calcium concentration (mg/100 mL) of finishing pigs fed control diet or vitamin D₃-supplemented diet

Day ^a	Control	Treated ^b	<i>P</i> > <i>F</i>
Day 0	10.3	10.4	0.62 ^c
Day 5	10.0	12.6	0.01

^aDay 0 refers to the day that vitamin D₃ feeding was initiated. Day 5 refers to 2 d after last feeding of vitamin D₃.

^bPigs were fed 500,000 IU of vitamin D₃ per day for 3 d.

^cProbability of differences between 0 and 5 d for each group.

the pork. On the basis of data from Experiments 1 and 2, we decided to feed 500,000 IU vitamin D₃ per d for 3 d to investigate the effect that supplemental dietary vitamin D₃ would have on pork-loin quality and tenderness.

Experiment 3

Plasma calcium concentrations of pigs fed the control diet or diet supplemented with 500,000 IU of vitamin D₃ for 3 d are shown in Table 1. Plasma calcium was similar (*P* = 0.62) initially (0 d), but the vitamin D₃-fed pigs exhibited greater (*P* < 0.01) plasma calcium concentration on the day of slaughter (5 d). The increase in plasma calcium for the treated pigs was 21%.

Body weight and carcass traits of control and vitamin D₃-supplemented pigs are shown in Table 2. No differences (*P* > 0.05) were observed for weight gain from 0 d to 5 d of the feeding trial when control and treated groups were compared. Furthermore, no differences were observed between the groups for hot carcass weights and dressing percentages. The carcasses from vitamin D₃-fed pigs, however, were lighter in weight than those of control pigs (*P* < 0.05), indicating greater water loss during chilling.

Subjective quality scores for color, marbling, and firmness were not different for treatment groups (Table 3). In contrast, Enright et al. (1998) reported a linear increase in longissimus color scores of pork loins with

Table 3. Effect of dietary vitamin D₃ on subjective measures of quality of loin chops at 10 to 11th rib

Item	Control	Treated	<i>P</i> > <i>F</i>
Color ^a	1.73	1.82	0.68
Firmness ^b	2.55	2.91	0.27
Marbling ^c	2.09	2.09	1.0

^aColor: 1—pale, pinkish gray; 5—dark, purplish red.

^bFirmness: 1—very soft and watery; 5—very firm and dry.

^cMarbling: 1—practically devoid; 5—moderately abundant or greater.

increasing supplemental dietary vitamin D₃ for market pigs.

To evaluate effect of supplemental vitamin D₃ on visual qualities of pork, Hunter score values of loins from control and vitamin D₃-supplemented pigs were determined. Hunter color data were not different for L*, a*, and b* values for any measured time period before and including 24-h postmortem (Table 4). At only 7 d and 14 d postmortem, L* values were lower (*P* < 0.02 and *P* < 0.01, respectively) for vitamin D₃-treated pigs. Vitamin D₃ supplementation did not influence L* values at earlier (<24 h) and later (21 d) times. The a* values were higher (*P* < 0.03) for loins at only 14-d postmortem from vitamin D₃-treated pigs. These L* and a* values correspond to a darker (L*) and redder (a*) loin color. No differences between groups were observed for b* values of loins assayed at any of the times up to 21-d postmortem. Enright et al. (1998) also reported lower L* values for loins from vitamin D₃-treated pigs. Darker-colored loin chops may be of higher value to Asian consumers (Miller and Lloyd, 1998).

Data for WHC and pH of loin chops (stored for 1, 7, 14, and 21 d at 2°C) from control and vitamin D₃-fed pigs are shown in Table 5. No differences (*P* < 0.05) because of vitamin D₃ supplementation were observed of loin chops stored at 2°C up to 21 d for WHC and pH values. Furthermore, pH values were at or above values where one might expect pork-quality problems (e.g., pale, soft, exudative pork) to develop (Bendall and Swatland, 1989). In contrast to our results, Enright et al. (1998) reported a decrease in drip-loss and an increase in WHC of pork because of dietary vitamin D₃ supplementation. The differences between their study and ours may be the length of time of vitamin D₃ supplementation; they supplemented the pigs for 10 d compared with 3 d in our study.

The effect of supplemented dietary vitamin D₃ on tenderness of loin chops was determined by the Warner-Bratzler shear force method and by the Star-Probe puncture method. Results from both measures of tenderness indicated that feeding 500,000 IU of vitamin D₃ to pigs for 3 d (3 to 5 d before slaughter) did not improve tenderness of loin chops (*P* > 0.05, Table 6). Research with beef cattle has shown that plasma calcium increases of 25% were associated with increased longissimus proteolysis and subsequently a more tender product (Swanek et al., 1999; Montgomery et

Table 2. Body weight and carcass characteristics of pigs fed control diet or vitamin D₃-supplemented diet

Item	Control	Treated ^a	<i>P</i> > <i>F</i> ^b
Live weight			
Day 0 ^c kg	117.4	117.2	0.31
Day 5 ^c kg	121.9	119.3	0.87
Gain, kg	4.5	2.1	0.12
Hot carcass weight, kg	89.2	87.2	0.21
Dressing %	73.0	71.9	0.10
Chilled carcass weight, kg	87.3	84.3	0.05

^aPigs were fed 500,000 IU of vitamin D₃ per day for 3 d.

^bProbability of differences between traits of control and treated pigs or their carcasses.

^cDay 0 refers to the day that vitamin D₃ feeding was initiated. Day 5 refers to 2 days after last feeding of vitamin D₃.

^dChilled at 2°C for 24 h.

Table 4. Hunter color values of longissimus from pigs fed control diet or vitamin D₃-supplemented diet

Hunter color	Time postmortem ^a	Control	Treated ^b	<i>P</i> > <i>F</i>
L*	24 h	40.0	39.2	0.52
	7 d	40.1	38.6	0.02
	14 d	39.6	38.4	0.01
	21 d	39.6	39.2	0.56
a*	24 h	13.9	14.1	0.47
	7 d	13.7	14.0	0.36
	14 d	13.4	14.0	0.03
	21 d	13.8	14.3	0.21
b*	24 h	6.5	6.3	0.53
	7 d	6.8	6.6	0.18
	14 d	6.7	7.3	0.47
	21 d	7.1	6.9	0.55

^aLongissimus samples were stored for indicated times at 2°C.

^bPigs were fed 500,000 IU of vitamin D₃ per day for 3 d.

al., 2000). It is possible that the intracellular calcium concentration of loin chops may not have become elevated above control values as a result of feeding vitamin D₃ to pigs as has been shown for beef (Swanek et al., 1999). Our hypothesis was that the greater extracellular calcium concentration (Table 1) would have increased the intracellular muscle calcium concentrations sufficiently to result in greater calpain activity, greater proteolysis, and thus greater tenderness (Huff-Lonergan et al., 1996). Another possibility is that calpain activity in loin chops is nonlimiting regarding tenderization during postmortem aging of loin chops. Furthermore, there may need to be a greater plasma calcium increase in pigs to be able to observe an increase in intracellular muscle calcium during postmortem aging and a subsequent improvement in proteolysis and postmortem tenderization. This possibility cannot be addressed by the current study, because intracellular muscle calcium concentrations were not determined.

Perhaps feeding the 500,000 IU of vitamin D₃ for longer times before slaughter or feeding greater daily dosages of vitamin D₃ would have resulted in an improvement in loin-chop tenderness. Feeding greater dosages of vitamin D₃ for less than 3 d before slaughter may be most practical to avoid possible decreases in feed intake because of the negative impact of dietary vitamin D₃ on rate of gain (Enright et al., 1998).

Implications

Although the objective of improving pork tenderness with high doses of dietary vitamin D₃ was not realized, in this study, the data regarding pork-quality improvements were encouraging. The changes in L* and a* values at 7 and 14 d postmortem have potential in meeting pork export demands, especially in the Asian market. Future research should focus on time and dose relationships of dietary vitamin D₃ and on the effect

Table 5. Water-holding capacity and pH of loin chops from pigs fed control diet or vitamin D₃-supplemented diet

Item	Days postmortem ^a	Control	Treated ^b	<i>P</i> > <i>F</i>
Water-holding capacity	1	3.24	2.86	0.12
	7	2.86	2.73	0.19
	14	3.03	3.21	0.71
	21	3.20	2.97	0.42
pH	1	5.53	5.60	0.15
	7	5.79	5.79	0.93
	14	5.77	5.70	0.28
	21	5.80	5.76	0.25

^aLongissimus samples were stored for indicated times at 2°C.

^bPigs were fed 500,000 IU of vitamin D₃ per day for 3 d.

Table 6. Warner-Bratzler shear forces and Star probe values of loin chops from pigs fed control diet or vitamin D₃-supplemented diet

Item	Days postmortem ^a	Control	Treated ^b	<i>P</i> > <i>F</i>
Warner-Bratzler Shear, kg				
	1	3.57	3.65	0.82
	7	3.10	3.06	0.88
	14	3.01	3.06	0.83
	21	2.92	3.04	0.51
Star probe, kg				
	1	5.73	5.92	0.58
	7	5.26	5.65	0.54
	14	5.71	5.46	0.33
	21	5.04	5.20	0.31

^aLongissimus samples were stored for indicated times at 2°C.^bPigs were fed 500,000 IU of vitamin D₃ per day for 3 d.

of dietary vitamin D₃ on muscle calcium partitioning and proteolysis.

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